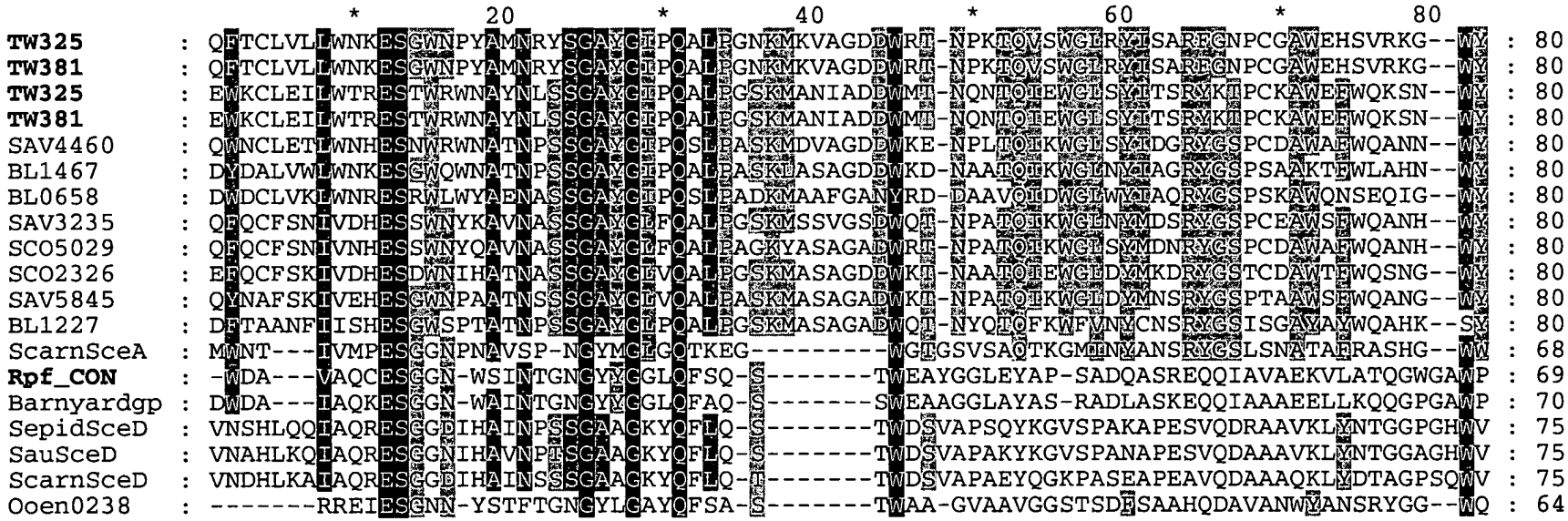
EXHIBIT A

Figure 1: Multiple sequence alignment of the Rpf-like domain of *Tropheryma whipplei* proteins with other similar proteins



SauSceD = *S. aureus* SceD, SepidSceD = *S. epidermidis* SceD, ScarnSceD = *S. carnosus* SceD, ScarnSceA = *S. carnosus* SceA, **Rpf_CON = Rpf domain consensus sequence** and Barnyardgp = Phage barnyard gp33 tape measure protein (Pedulla et al., Cell, 113, 171-182). TW389 & TW 442 have been sequenced from two different strains of *T whipplei* (TW08/27 and Twist). Other genes are from *Streptomyces avermitilis* (SAV), *Streptomyces coelicolor* (SCO) and *Bifidobacterium longum* (BL). The shading represents the degree of conservation at any one position within the alignment. Residues in black are 100 % conserved, those in dark grey are 80 % conserved, those in light grey are 60 % conserved and those in white are conserved in less than 60 % of sequences.

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Figure 2: Individual alignments between TW325 and other Rpf-like proteins in Fig. 1A

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                  *        20         *        40         *        60         *        80
TW325     : QFTCLVLLWNKESGWNPYAMNRYSGAYGIPQALPGNKMKVAGDDWRTNPKTQVSWGLRYISARFGNPCGAWEHSVRKG---WY
MlutRpf     TWDR---LAECESNGT-WDINTGNGFYGGVQFTLS--------SWQAVGGEGY-P---HQASKAEQIKRAEILQDLQGWGAWPLCS
             :     *   **    :  *   * **  *             *                  :  :   *       *   *

TW325     : QFTCLVLLWNKESGWNPYAMNRYSGAYGIPQALPGNKMKVAGDDWRTNPKTQVSWGLRYISARFGNPCGAWEHSVRKG---WY
Rv1009      IWDA---IAGCEAGGN-WAINTGNGYYGGVQFDQG--------TWEANGGLRYAP-RADLATREEQIAVAEVTRLRQGWGAWPVCA
(z94752)     :     :   * * * :* *   * **  *   *          *  *           :  *  :   *    :* *   *

TW325     : QFTCLVLLWNKESGWNPYAMNRYSGAYGIPQALPGNKMKVAGDDWRTNPKTQVSWGLRYISARFGNPCGAWEHSVRKG---WY
Rv1884c     NWDA---VAQCESGGN-WAANTGNGKYGGLQFKPA--------TWAAFGGVGN-P---AAASREQQIAVANRVLAEQGLDAWPTCGA
(U38939)    ::     : : *** * :* *   * **  *  *          *                  *  :   *       *   *

TW325     : QFTCLVLLWNKESGWNPYAMNRYSGAYGIPQALPGNKMKVAGDDWRTNPKTQVSWGLRYISARFGNPCGAWEHSVRKG---WY
Rv2389c     DWDA---IAQCESGGN-WAANTGNGLYGGLQISQA--------TWDSNGGVGS-P---AAASPQQQIEVADNIMKTQGPGAWPKCSS
(z81368)     :     : : *** * :* *   * **  *             * :*                  :   *       *   *

TW325     : QFTCLVLLWNKESGWNPYAMNRYSGAYGIPQALPGNKMKVAGDDWRTNPKTQVSWGLRYISARFGNPCGAWEHSVRKG---WY
Rv2450c     NWDA---IAQCESGGN-WSINTGNGYYGGLRFTAG--------TWRANGGSGSA----ANASREEQIRVAENVLRSQGIRAWPVCGR
(MTV008)    ::     : : *** * :  *   * **      *          ** *                *  :   *       *   *

TW325     : QFTCLVLLWNKESGWNPYAMNRYSGAYGIPQALPGNKMKVAGDDWRTNPKTQVSWGLRYISARFGNPCGAWEHSVRKG---WY
Rv0867c     EWDQ---VARCESGGN-WSINTGNGYLGGLQFTQS--------TWAAHGGGEFAP-SAQLASREQQIAVGERVLATQGRGAWPVCGR
(MTV043)     :     :   *** * :  *   *  *  *             *                * *  :           *   *

TW325     : QFTCLVLLWNKESGWNPYAMNRYSGAYGIPQALPGNKMKVAGDDWRTNPKTQVSWGLRYISARFGNPCGAWEHSVRKG---WY
Rpf_CON      WDA---VAQCESGGN-WSINTGNGYYGGLQFSQS--------TWEAYGGLEYAP-SADQASREQQIAVAEKVLATQGWGAWP
             :     : : *** * :  *   * **  * :          *                  *  :   *       *   *
```

	Rpf	Rv0867c	Rv1009	Rv1884c	Rv2389c	Rv2450c	Rpf consensus
% Identity (similarity) N-terminus (i.e. residues 1-35)	22.8 (28.6)	22.8 (31)	28.6 (37.1)	31.4 (45.7)	28.6 (40)	25.7 (40)	25.7 (40)
% Identity (similarity) complete domain (i.e. residues 1-80)	15 (20)	16.3 (22)	21.3 (28.8)	20 (27.5)	18.8 (26.3)	20 (27.5)	17.5 (25)

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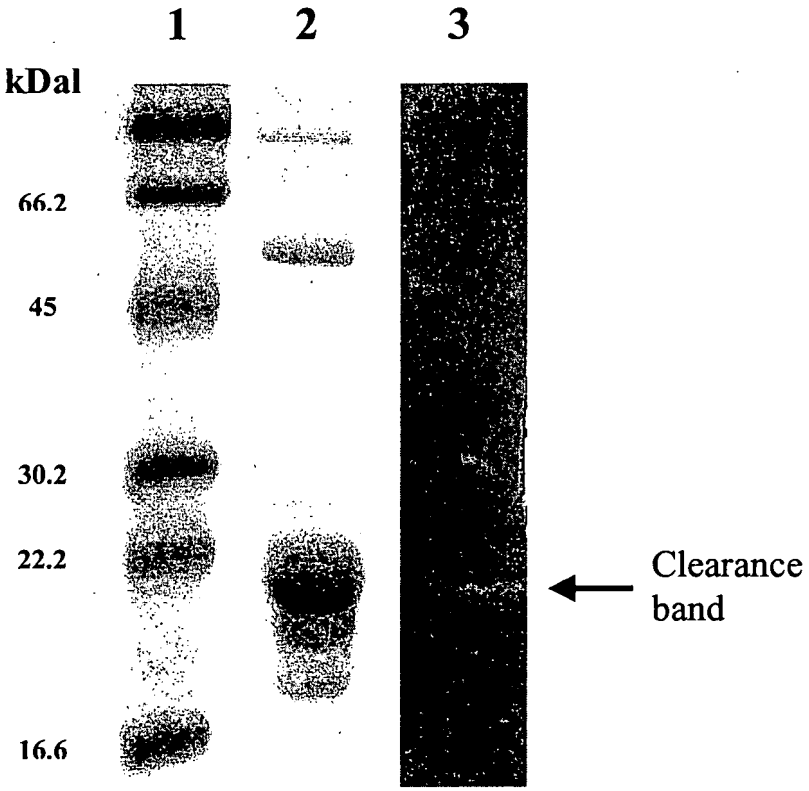
```
                  *        20         *        40         *        60         *        80
TW325     : QFTCLVLLWNKESGWNPYAMNRYSGAYGIPQALPGNKMKVAGDDWRTNPKTQVSWGLRYISARFGNPCGAWEHSVRKG---WY : 80
Mlep104666 EWDQ---VARCESGGN-WSINTGNGYLGGLQFSQG--------TWASHGGGEYAP-SAQLATREQQIAVAERVLATQGSGAWPACG
            :     :   *** * :  *   *  *  *   *          * :            :  *  :   *       *   *

TW325     : QFTCLVLLWNKESGWNPYAMNRYSGAYGIPQALPGNKMKVAGDDWRTNPKTQVSWGLRYISARFGNPCGAWEHSVRKG---WY : 80
Mlep101095 NWDA---VAQCESGRN-WRANTGNGFYGGLQFKPT--------IWARYGGVG----NPAGASREQQITVANRVLADQGLDAWPKCGA
            ::     : : *** * :  *   * **  *  *          *                    *  :   *       *   *

TW325     : QFTCLVLLWNKESGWNPYAMNRYSGAYGIPQALPGNKMKVAGDDWRTNPKTQVSWGLRYISARFGNPCGAWEHSVRKG---WY : 80
Scoe6C12    WDA---IAACESSGN-WQANTGNGYYGGLQFA-R-------SSWIAAGGLKYAP-RADLATRGEQIAVAERLARLQGMSAW-GCA
            :    :    **  * :  *   * **  *             *              :  *  :   *       *   *
```

	Mlep104666	Mlep101095	Scoe6C12
% Identity (similarity) N-terminus (i.e. residues 1-35)	25.7 (34.3)	28.6 (42.9)	22.8 (31.4)
% Identity (similarity) complete domain (i.e. residues 1-80)	17.5 (25)	18.8 (26.3)	16.3 (22.5)

Figure 3 Zymogram showing muralytic activity of recombinant TW325 protein isolated from *E. coli* strain LMG194.



Lane 1 Size markers
Lane 2 Recombinant TW325
Lane 3 Zymogram corresponding to the protein in lane 2

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Figure 4: Experimental Detail

i) Data Demonstrating Related Activity for Rpf Proteins and TW325

Recent work has shown that the Rpf proteins have cell wall lytic activity (i.e. they are murein hydrolases). This has been demonstrated in several ways:

- activity staining in gels (zymograms);
- up to 50% loss of optical density of a suspension of *M. luteus* cell wall fragments during incubation with recombinant Rpf;
- release of diaminopimelic acid-containing material into the soluble fraction using fluorescent-labeled cell walls;
- the precise bond that is cleaved in the murein is under current investigation.

We have demonstrated that a recombinant form of one of the two *T. whipplei* proteins (TW325) has similar murein hydrolase activity using zymograms.

ii) Outline Experimental Detail

M. luteus cells grown in 1 litre of LB medium overnight (to stationary phase) were centrifuged at 10,000 g, washed with water, re-suspended in 200 ml 5% SDS and boiled for 20 minutes. Following centrifugation, the pellet was re-suspended in 100 ml of 4 % of SDS and boiled again. Then pellet was then washed 6 times with hot (65°C) water to remove SDS. It was finally washed with acetone, air-dried and stored at -20°C. Before use the pellet was resuspended in deionised water and passed through fine syringe needle to make a homogeneous suspension. Cell wall fragments were incorporated at a final concentration of 0.2% in zymogram gels.

Zymogram analyses were performed as described before (Lepeuple *et al.*, 1998). Different buffers with various pH and compositions were employed) to re-nature proteins after SDS-PAGE. 25 mM TrisCl buffer, pH 6.0 was found to be optimal. Following SDS-PAGE, gels were soaked in deionised water with gentle shaking for 20 min, then in a sample of renaturation buffer for 30 min. Finally gels were transferred into100 ml of fresh renaturation buffer and incubated at 30°C overnight (37°C for recombinant TW325). To improve contrast, gels were stained in 0.1 % of methylene blue in 0.01% KOH and de-stained as described before.

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